TECHNICAL MANUAL

Trehalose Colorimetric Assay Kit

- **SKU CODE:** MAES0435
- **SIZE:** 48T / 96T
- **DETECTION PRINCIPLE:** Colorimetric
- **RUO:** Research-Use-Only

Revised: 01/2026

Trehalose Colorimetric Assay Kit

Please read entire manual carefully before starting experiment.

Table of Contents

1. Key Features

Specification:

96T (40 samples)

Measuring instrument:

Microplate reader (550-520 nm)

Detection range:

0.007-1.00 mg/mL

Sample type:

Fungus, algae and plant tissue samples

2. Storage & Expiry

This product should be stored at -20°C in dark conditions for up to 12 months. For detailed storage instructions of individual kit components, please refer to Section 4. The expiration date is indicated on the outer label of the kit box.

3. Product Description

The Assay Genie Trehalose Assay Kit is designed for the quantitative determination of trehalose in biological samples. Trehalose is a non-reducing disaccharide composed of two glucose units and serves as an important energy reserve and stress-protective molecule in many lower plants, invertebrates, and microorganisms. Its unique stabilizing properties have also led to widespread applications across the food, cosmetic, pharmaceutical, and agricultural industries.

In this assay, trehalose is specifically hydrolyzed by trehalase to generate two molecules of glucose. The released glucose is subsequently quantified using the GOD-POD colorimetric method. A parallel control well is included to correct for endogenous glucose, ensuring accurate measurement of trehalose-derived glucose only. The assay offers high specificity, excellent sensitivity, and reliable performance across a range of sample types.

This dual function kit includes validated Bradford Reagent to quantify total protein concentration for accurate sample normalization.

4. Kit Contents

No	Component Name	Size (48T)	Size (96T)	Storage
1	Enzyme Reagent	0.3 mL ×1vial	0.6 mL×1vial	2-8℃,12 months (avoid light)
2	Chromogenic Agent A	6 mL×1vial	12 mL×1vial	2-8℃,12 months (avoid light)
3	Chromogenic Agent B	4.5 mL ×1vial	9 mL×1vial	2-8℃,12 months (avoid light)
4	Trehalose Standard	Powder × 1 vial	Powder×1vial	2-8℃,12 months (avoid light)
5	Microplate	48 wells	96 wells	-
6	Plate Sealer	2 pieces	2 pieces	-
7	Bradford Reagent	2 mL	2 mL	RT

Note: *All reagents must be stored according to the specified conditions listed in the table above. Do not mix reagents from different kits, as this may compromise assay performance. For reagents provided in small volumes, centrifuge briefly before use to ensure complete recovery of the contents.*

Additional materials required:

- **Instruments:** Microplate reader (500-520 nm, optimum wavelength: 510 nm), Incubator (37℃)

5. Important Notes

1. This assay kit is intended for Research Use Only. Assay Genie assumes no responsibility for any issues or legal liabilities arising from the use of this kit for clinical diagnostics or any other unauthorized purposes.
2. Please read the instructions carefully before beginning the assay. Ensure that all instruments are correctly calibrated. Strict adherence to the protocol is essential for accurate results.
3. Appropriate laboratory safety precautions must be followed, including the use of lab coats and latex gloves.
4. If the concentration of the target substance falls outside the detection range, please adjust the sample by performing further dilution or concentration as needed.
5. If your sample type is not listed in the instruction manual, we strongly recommend performing a preliminary test to confirm compatibility.
6. Experimental outcomes depend on multiple factors including reagent integrity, handling technique, and laboratory conditions. While Assay Genie guarantees the quality of our kits, we are not responsible for any loss of samples during use. We advise calculating sample requirements in advance and ensuring adequate sample volume is reserved before starting the assay.

6. Reagent Preparation

1. Equilibrate all reagents to 25℃ before use.
2. **Preparation of 10 mg/mL standard solution**: Dissolve one vial of trehalose standard with 1 mL of double distilled water, mix well to obtain the 10 mg/mL standard solution. Store at 2-8℃ for 1 month.
3. **Preparation of 1 mg/mL standard solution:** Before testing, please prepare sufficient 1 mg/mL standard solution according to the test wells. For example, prepare 50 µL of 1 mg/mL standard solution (mix well 5 µL of 10 mg/mL standard solution and 45 µL of double distilled water). The 1 mg/mL standard solution should be prepared on spot and used up within 8 hours.

7. Sample preparation

A. Sample Preparation

Tissue samples

1. Harvest the amount of tissue needed for each assay (initial recommendation 0.1 g).
2. Add tissue samples into 2 mL EP tube and heated in a 90℃ water bath for 10 min.
3. Add 0.9 mL of double distilled water into the tubes and homogenize with a dounce homogenizer at 4℃.
4. Centrifuge at 10000×g for 10 min at 25 ℃ to remove insoluble material. Collect supernatant and keep it on ice for detection

B. Dilution

Sample Type	Dilution factor
10% Pleurotus djamor pileus tissue homogenate	8-16
10% Pleurotus ostreatus pileus tissue homogenate	5-10
10% Lentinus edodes pileus tissue homogenate	2-4

Note: *The recommended diluent is double distilled water. For sample types not specified in the protocol, it is advised to perform a preliminary test to determine the appropriate dilution factor or contact our Tech Support Team at techsupport@assaygenie.com.*

7.1. Protein Quantification (Optional)

To quantify total protein levels, use the Bradford Reagent included in this kit. Visit Bradford Protein Assay Protocol to view the full protocol.

8. Assay Procedure

1. Sample well: Add 10 µL of sample to sample wells. Control well: Add 10 µL of sample to control wells. Standard well: Add 10 µL of 1 mg/mL standard to standard wells.
2. Add 10 µL of enzyme reagent to sample and standard wells.
3. Add 100 µL of chromogenic agent A to sample and standard wells. Add 110 µL of chromogenic agent A to control wells. Add 120 µL of chromogenic agent A to blank wells.
4. Add 80 µL of chromogenic agent B to eachwell.
5. Mix fully with microplate reader for 5 s and incubate at 37°C for 30 min protected from light. Measure the OD value of each well at 510 nm, as A.

9. Data Analysis

Tissue Sample

$$\text{Trehalose content (mg/g wet weight)} = \frac{A_{sample} - A_{control}}{A_{standard} - A_{blank}} \times c \times f \div \frac{m}{V}$$

Note:

c: The concentration of standard, 1 mg/mL.

f: Dilution factor of sample before test.

m: The wet weight of sample, g.

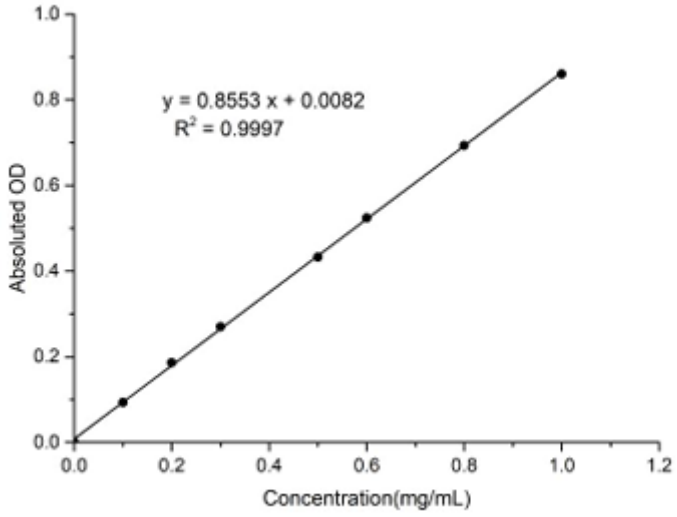
V: The volume of double distilled water in the preparation step of tissue, mL.6.

10. Typical Data

Standard Curve

The OD values of the standard curve may vary depending on specific assay conditions, such as operator technique, pipetting accuracy, and temperature fluctuations. Therefore, the standard curve and data provided below are for reference only and should not be used for direct result calculation. Always generate a fresh standard curve for each assay run.

Concentration (mg/mL)	0	0.1	0.2	0.3	0.5	0.6	0.8	1.0
OD Value	0.054	0.146	0.235	0.321	0.485	0.579	0.742	0.912
	0.055	0.151	0.247	0.329	0.491	0.579	0.753	0.918
Average OD	0.055	0.148	0.241	0.325	0.488	0.579	0.748	0.915
Normalized OD	0.000	0.093	0.186	0.270	0.433	0.524	0.693	0.860



Sensitivity

The analytical sensitivity of the assay is 0.007 mg/mL. This value was determined by measuring the zero standard (blank) in 20 independent replicates, calculating the mean OD and adding two standard deviations. The corresponding concentration was then derived from the standard curve.

Recovery

Three sample concentrations (high, medium, and low) were tested in parallel, with six replicates per concentration. The average recovery rate across all concentrations was determined to be 98%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (mg/mL)	0.20	0.50	0.80
Observed Conc. (mg/mL)	0.19	0.50	0.78
Recovery rate (%)	97	100	97

Intra-assay Precision

Three pleurotus djamor pileus tissue samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Parameters	Sample 1	Sample 2	Sample 3
Mean (mg/mL)	0.2	0.5	0.8
%CV	1.3	2.6	1.8

Inter-assay Precision

Three pleurotus djamor pileus tissue samples were assayed 20 times in duplicate by three operators to determine precision between assays.

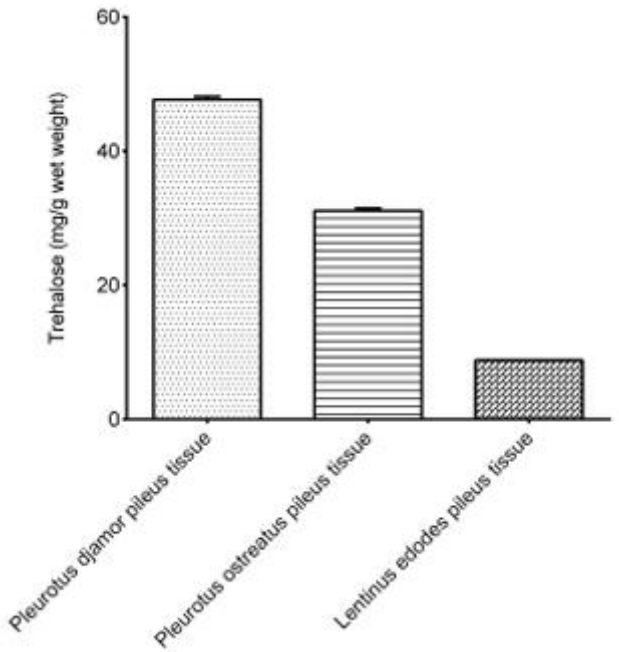
Parameters	Sample 1	Sample 2	Sample 3
Mean (mg/mL)	0.20	0.50	0.80
%CV	6.6	3.6	1.9

11. Example Analysis

Take 10 μL of 10% pleurotus ostreatus pileus tissue homogenate which dilute for 6 times and carry the assay according to the operation steps. The results are as follows: The OD value of the sample well is 0.546, the OD value of the control well is 0.054, the OD value of the standard well is 0.908, the OD value of the blank well is 0.053, and the calculation result is:

Trehalose content (mg/g wet weight) = (0.546- 0.054) ÷ (0.908- 0.053) × 1 × 6 ÷ 0.1 × 0.9 = 31.07 mg/g wet weight

Detect 10% pleurotus djamor pileus tissue homogenate (dilute for 8 times), 10% pleurotus ostreatus pileus tissue homogenate (dilute for 6 times) and 10% lentinus edodes pileus tissue homogenate (dilute for 2 times) according to the protocol, the result is as follows:



Notes:

Notes:

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.